



# Effect of low-dose treatment with selegiline on dopamine transporter (DAT) expression and amphetamine-induced dopamine release *in vivo*

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**1** Chronic treatment with low doses of the selective monoamine oxidase (MAO) type B inhibitors selegiline [(–)-deprenyl] and rasagiline, causes elevation in extracellular level of 3,4-dihydroxyphenylethylamine (dopamine) in the rat striatum *in vivo* (Lamensdorf *et al.*, 1996). The present study was carried out to determine whether this effect of selegiline could be the result of an inhibition of the high-affinity dopamine neuronal transport process.

**2** Changes in activity of the dopamine transporter (DAT) *in vivo* following selegiline treatment were evaluated indirectly by microdialysis technique in the rat, from the change in striatal dopamine extracellular concentration following systemic amphetamine administration (4 mg kg<sup>–1</sup>, i.p.). Striatal levels of the DAT molecule were determined by immunoblotting. Uptake of [<sup>3</sup>H]-dopamine was determined in synaptosomes from selegiline-treated animals.

**3** Amphetamine-induced increase in striatal extracellular dopamine level was attenuated by one day and by chronic (21 days) treatment with selegiline (0.25 mg kg<sup>–1</sup>, s.c.).

**4** Striatal levels of DAT were elevated after 1 and 21 days treatment with selegiline, but were not affected by clorgyline, rasagiline, nomifensine or amphetamine.

**5** The increase in DAT expression, and attenuation of amphetamine-induced dopamine release, were not accompanied by a change in [<sup>3</sup>H]-dopamine uptake in synaptosomes of selegiline-treated animals.

**6** The results suggest that a reversible inhibition of dopamine uptake occurs following chronic low dose selegiline treatment *in vivo* which may be mediated by an increase in endogenous MAO-B substrates such as 2-phenylethylamine, rather than by the inhibitor molecule or its metabolites. Increased DAT expression appears to be a special property of the selegiline molecule, since it occurs after one low dose of selegiline, and is not seen with other inhibitors of MAO-A or MAO-B. The new DAT molecules formed following selegiline treatment appear not to be functionally active.

**Keywords:** Selegiline; (–)-deprenyl; rasagiline; clorgyline; nomifensine; dopamine uptake; dopamine transporter; microdialysis; monoamine oxidase; amphetamine

**Abbreviations:** MAO, monoamine oxidase; dopamine, 3,4-dihydroxyphenylethylamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic; DAT, dopamine transporter; EDTA, ethylenediaminetetracetic acid; HSA, heptane-sulphonic acid; PBS, phosphate-buffered saline; PEA, 2-phenylethylamine

## Introduction

In the rat, striatal extracellular level of 3,4-dihydroxyphenylethylamine (dopamine) as determined by *in vivo* microdialysis is enhanced by selective inhibition of monoamine oxidase type A (MAO-A), but unaffected by inhibition of MAO-B, when the inhibitors are administered acutely (Arbuthnott *et al.*, 1990; Butcher *et al.*, 1990; Kato *et al.*, 1986). This is consistent with the fact that the isoform of MAO associated with dopaminergic neurons of the nigro-striatal tract in primates and rodent species is primarily type A (Demarest *et al.*, 1980; Fagervall & Ross, 1986; Westlund *et al.*, 1985).

In a previous microdialysis study in the rat, we observed that chronic (21 days) treatment with a low, MAO-B selective dose of selegiline [(–)-deprenyl] or rasagiline [(R(+))N-propargyl-1-aminoindan]; a selective MAO-B inhibitor which is not metabolized to amphetamine-like metabolites, increased striatal dopamine extracellular level (Lamensdorf *et al.*, 1996). The pattern of changes obtained after repeated treatment with both MAO-B inhibitors (i.e., elevation in

dopamine extracellular level without significant effect on 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) levels is similar to that obtained after treatment with classical uptake blockers like cocaine and nomifensine (Hurd & Ungerstedt, 1989). Chronic administration of a low dose of selegiline also enhanced dopamine release from rat striatal slices *in vitro* (Dluzen & McDermott, 1991; Knoll, 1985; 1992) and inhibited dopamine re-uptake in striatal slices (Zsilla *et al.*, 1986; Tekes *et al.*, 1988). The uptake inhibition seen after repeated selegiline administration was reversible, since the degree of inhibition was noticeably less 24 h after the last dose (Zsilla *et al.*, 1986) by contrast with the inhibition of MAO, which is irreversible.

The effect of selegiline on dopamine release could be the result of an amphetamine-like action, either of the parent molecule or metabolites, because selegiline is metabolized to (–)-methamphetamine and (–)-amphetamine (Reynolds *et al.*, 1978). However, rasagiline is not metabolized to amphetamine-like metabolites but to 1-aminoindane which does not possess amphetamine-like properties (Finberg *et al.*, 1998). As a result, we proposed (Lamensdorf *et al.*, 1996) that

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the apparent uptake-inhibitory properties of selegiline and rasagiline could be the result of the action of the MAO-B substrate 2-phenylethylamine (PEA), tissue levels of which increase markedly after selegiline treatment (Paterson *et al.*, 1990).

In the current study, we further investigated the effect of selegiline on the dopamine transporter. The activity of the dopamine transporter *in vivo* was assessed from the increase in extracellular fluid dopamine caused by systemically-administered amphetamine. In addition, the action of selegiline and rasagiline was studied on [ $^3$ H]-dopamine uptake in striatal synaptosomes, and the effect of MAO inhibition and amphetamine on dopamine transporter protein levels was evaluated by immunoblotting.

## Methods

### Animals

Adult male Sprague-Dawley rats were housed in wire mesh cages at an environmental temperature of 19–21°C, 12 h light-dark cycle, and were fed solid rat chow and water *ad libitum* for the duration of the experiment. The rats weighed between 320–340 g at the time of dialysis probe implantation or sacrifice. All drugs were administered by s.c. or i.p. injection. Animal procedures were authorized by the Technion Animal Care and Use Committee.

### Drug treatment

For comparison between the effects of chronic and acute selegiline treatment, rats received 0.25 mg kg<sup>-1</sup> selegiline daily s.c. for 21 days (chronic), or 20 days saline and 0.25 mg kg<sup>-1</sup> selegiline on day 21 (acute), or saline daily for 21 days (control). On day 21, a microdialysis probe was implanted into the left striatum (see below), and sampling of dialysate was carried out in the awake animals on day 22 (24 h after last dose of saline or drug).

Dopamine transporter (DAT) levels were determined in rats treated with selegiline (0.25 mg kg<sup>-1</sup>, s.c.) or rasagiline (0.05 mg kg<sup>-1</sup>, s.c.) for 1 or 21 days as above, as well as in rats which received 21 days of saline treatment and drug on day 22, 2 h before decapitation. Striatal DAT level was also determined after 21 days of repeated clorgyline administration (0.2 mg kg<sup>-1</sup>, s.c.) and 1 day after nomifensine (20 mg kg<sup>-1</sup>, i.p.) or ( $\pm$ )-amphetamine (0.25 mg kg<sup>-1</sup>, s.c.).

Inhibition of [ $^3$ H]-dopamine uptake by the MAO inhibitors and their metabolites was determined in striatal synaptosomes prepared from untreated rats. Kinetics of [ $^3$ H]-dopamine uptake were determined in striatal synaptosomes prepared from rats which had been treated once or 21 days with selegiline and killed 24 h after the last dose as described above.

### Microdialysis procedure

Dialysis probes (4 mm active length) were positioned under stereotaxic guidance in the striata of pentobarbital/chloralhydrate (12/60 mg kg<sup>-1</sup>, i.p.) anaesthetized rats, using the coordinates A 1.2, L 2.5, V 7.0 (Paxinos & Watson, 1982) and were perfused continuously with artificial CSF of composition (in mM): NaCl 150, CaCl<sub>2</sub> 1.7, KCl 3 and MgCl<sub>2</sub> 0.9 at 2  $\mu$ l min<sup>-1</sup>. Dialysate collections (20 min) were made in miniature polyethylene vials containing 10  $\mu$ l 0.1 N HClO<sub>4</sub>. Four to five control microdialysis collections were made, and then ( $\pm$ )-amphetamine (4 mg kg<sup>-1</sup>, i.p.) was administered. A

further six collections were then made. After the dialysis experiments, the placement of the probe was verified by visual inspection.

Microdialysis probes were fabricated from ceramic and stainless steel tubings, with a cuprophane membrane. The relative recovery for dopamine, DOPAC and HVA determined *in vitro* before implantation was 22.8  $\pm$  2.5, 13.2  $\pm$  1.9 and 15.2  $\pm$  3.5% (mean  $\pm$  s.e.mean of  $n=6-7$  determinations), respectively.

### Analysis of dialysate

Dialysates were analysed for dopamine, DOPAC and HVA by HPLC with electrochemical detection. Separation of dopamine and metabolites was achieved using a Microsorb column (Rainin C-18, 3  $\mu$ m, 4.6 mm diameter, 10 cm long) with mobile phase composed of 4.83 g NaHPO<sub>4</sub>, 90 mg HSA, 20 ml methanol, 10 ml acetonitrile and 50 mg EDTA per 500 ml HPLC grade deionized water. The flow rate through the system was 1.5 ml min<sup>-1</sup>. Compounds were detected with an ESA Coulochem model 5011 detector (ESA, U.S.A.) operated in redox mode. Column eluates were initially oxidized at a potential of +300 mV using an ESA guard cell placed before the detector, reduced to +60 mV at detector 1 and measured at -350 mV at detector 2. Chromatograms were analysed for peak height and retention time using an integrating recorder (Varian model 4270).

### Determination of dopamine transporter (DAT) levels by immunoblotting

Synthetic peptides, SHGIDDLGPPR and LTNSTLINPPQ-TPVEAQE, corresponding to residues 227–237 and 42–59 of the cloned human and rat DAT respectively, were synthesized by the Department of Biological Services, Weizmann Institute, Rehovot, Israel. Conjugation and immunization procedures were identical for each of the two peptides (Simantov *et al.*, 1996). Briefly, the peptide was conjugated to keyhole limpet haemocyanin, using glutaraldehyde, mixed with an equal volume of complete Freund adjuvant, and injected into two New-Zealand White rabbits in multisite intradermal injections. Additional boosts were given at 2–3 week intervals, and serum was prepared one and a half months after the last injection.

Dissected striata were homogenized in 2% sodium dodecyl sulphate, and protein levels were determined using the Lowry assay (Lowry *et al.*, 1951). Samples of 200  $\mu$ g protein were run on 7% sodium dodecyl sulphate-polyacrylamide gel, and blotted to nitrocellulose membrane. The membrane was preincubated at room temperature with 90% phosphate-buffered saline (PBS)-10% milk for 3 h, washed with PBS containing 0.05% Tween 20, and incubated with anti-DAT antibodies for 90 min. The membrane was washed, incubated for 2 h at room temperature with [ $^{125}$ I]-protein A, exposed to BAS IP screen ( $\llcorner$ Fugix $\gg$ ), scanned, and analysed using MacBAS 2.0 (Fugi, Fugix Bas 1000) software. The density of DAT immunoreactive band was determined by a phospho-imaging analyser, after subtracting the background level. A standard curve of 25–300  $\mu$ g protein from rat striatum was blotted in parallel to the samples, and was used to convert data to absolute values.

### [ $^3$ H]-dopamine uptake in rat striatal synaptosomes

Measurement of IC<sub>50</sub> values for inhibition of [ $^3$ H]-dopamine uptake into rat striatal synaptosomes by rasagiline, R-(+)-aminoindane, selegiline, (-)-desmethylselegiline, (-)-methamphetamine, nomifensine and GBR-12909 were carried

out according to Cooper & Carlson (1983) with slight modifications. Rats were sacrificed by decapitation, and striata homogenized in ice cold isotonic sucrose (0.32 M). Synaptosomes were incubated in Krebs'-Henseleit buffer (in mM): NaCl, 122;  $\text{NaH}_2\text{PO}_4$ , 15.8; KCl, 4.9;  $\text{CaCl}_2$ , 1.27;  $\text{MgSO}_4$ , 1.19; dextrose 11; pargyline 0.09; ascorbate 1.23; adjusted to pH 7.4 for 5 min before addition of [ $^3\text{H}$ ]-dopamine (100 nM). The incubation was terminated after 5 min by dilution and filtration under reduced pressure over glass fibre filters (Whatman GF/B). The filters were washed, and radioactivity determined by liquid scintillation spectrometry. Active uptake was calculated as difference between uptake at 37 and 0°C.

### Data analysis

Statistical analysis of difference between drug-treated animals in microdialysis experiments was performed by one-way analysis of variance (ANOVA). Probability values ( $P$ ) were calculated using Tukey-Kramer test.

The  $\text{IC}_{50}$  values for [ $^3\text{H}$ ]-dopamine uptake, and the area under the amphetamine microdialysis curve values were calculated using appropriate software (Graphpad Inplot, version 4.03).

### Materials

Selegiline hydrochloride, rasagiline, (–)-desmethylosegiline, (–)-methamphetamine, R-(+)-aminoindane and amphetamine sulphate were gifts of Teva (Israel). GBR-12909 and nomifensine were obtained from RBI (U.S.A.), [ $^3\text{H}$ ]-dopamine (specific activity  $20.34 \text{ Ci mmol}^{-1}$ ) from New England Nuclear-Dupont (England), and [ $^{125}\text{I}$ ]-Protein A (specific activity  $100 \mu\text{Ci ml}^{-1}$ ) from Amersham. All other reagents and chemicals were analytical grade and were purchased from either Sigma or Merck.

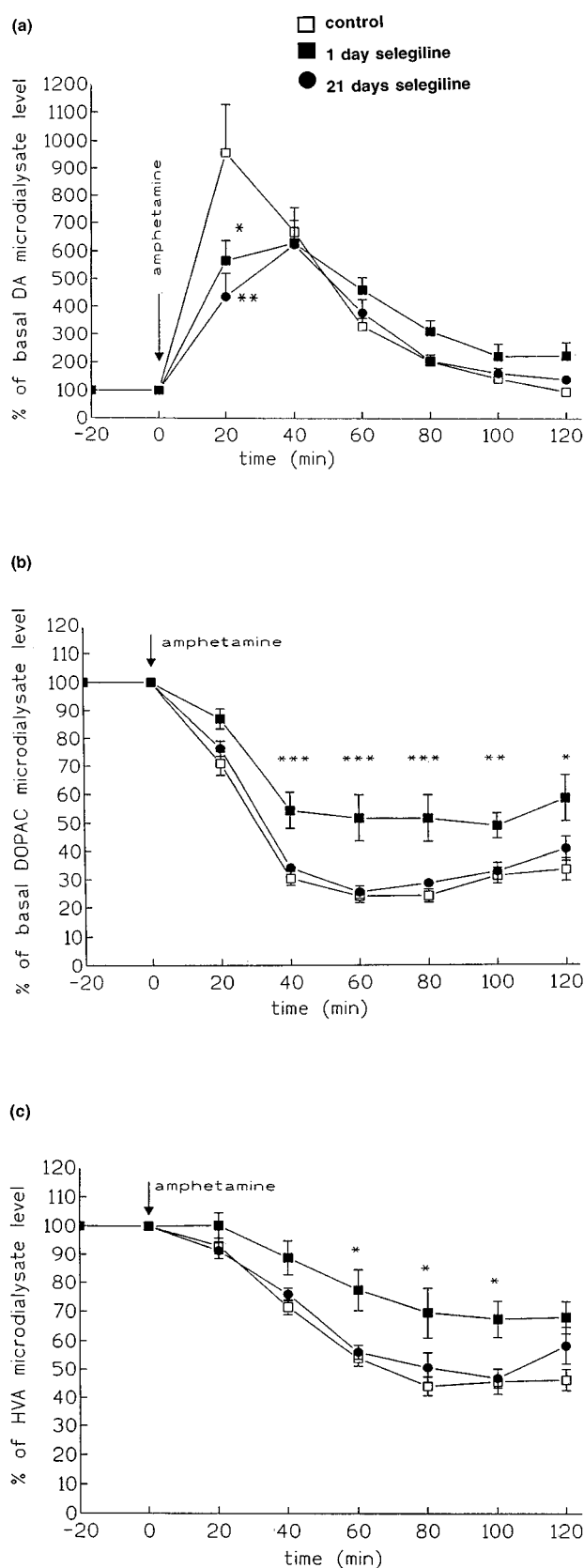
## Results

### Effects of amphetamine administration on striatal microdialysate levels of dopamine, DOPAC and HVA in rats treated with selegiline

Rats treated chronically with selegiline had a significantly higher dopamine basal extracellular level compared to the control and 1 day treated animals (see legend to Figure 1a) as we reported previously (Lamensdorf *et al.*, 1996). Chronic (21 days), and acute (1 day) treatment with selegiline ( $0.25 \text{ mg kg}^{-1}$ ) delayed (40 min vs 20 min, selegiline treated rats vs saline treated respectively), and significantly ( $P < 0.05$ ) attenuated the maximum dopamine release induced by amphetamine administration (Figure 1a). In the chronically, but not in acutely treated rats, area under the dopamine curve was significantly reduced ( $P < 0.05$  in comparison with acute selegiline and saline treated rats). Amphetamine-induced reduction in DOPAC and HVA microdialysate level was also attenuated 24 h after a single selegiline treatment although no changes were seen in the chronically treated rats (Figure 1b and c).

### Effects of selegiline treatment on DAT levels

Selegiline increased DAT level by 154 and 145% at 24 h and 21 days treatment respectively, without effect at 2 h (Table 1). Acute or chronic treatment with rasagiline did not significantly affect DAT level, nor did acute (24 h) treatment with



**Figure 1** (a–c) Effect of acute and chronic treatment with selegiline ( $0.25 \text{ mg kg}^{-1}$ , s.c.) on amphetamine ( $4 \text{ mg kg}^{-1}$ , i.p.) induced changes in striatal microdialysate levels of dopamine (a), DOPAC (b) and HVA (c). Amine and metabolite levels are given as mean  $\pm$  s.e. mean of per cent changes from basal levels. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  for difference compared to saline control group. Basal microdialysate levels for dopamine, DOPAC and HVA were: control (9)  $0.11 \pm 0.02$ ,  $38.3 \pm 3.6$ ,  $23.6 \pm 1.3$ ; acute (13)  $0.11 \pm 0.01$ ,  $32.16 \pm 3.76$ ,  $23.04 \pm 3.5$ ; chronic (12)  $0.21 \pm 0.02$ ,  $25.1 \pm 1.06$ ,  $16.7 \pm 0.9 \text{ pmol } 20 \text{ min}^{-1}$  respectively.

**Table 1** Effect of *in-vivo* drug treatments on striatal DAT levels

	Control	2 h	24 h	21 days
Selegiline	210 ± 21 (11)	174 ± 50 (3)	324 ± 13 (11)*	306 ± 31 (9)*
Rasagiline	210 ± 24 (9)	268 ± 70 (3)	226 ± 39 (9)	172 ± 23 (9)
Clorgyline	210 ± 24 (9)	NT	NT	208 ± 62 (6)
Amphetamine	83 ± 4 (4)	NT	72 ± 7 (4)	NT
Nomifensine	83 ± 4 (4)	NT	65 ± 7 (4)	NT

The intensity of the immunoreactive band corresponding to DAT was determined by the phosphoimaging analyser, and a standard curve of rat striatal membranes (0–300 µg protein) was used to extrapolate the band intensity to µg protein. Data shown are mean values (µg DAT ± s.e.mean) per striatal sample. Number of rats shown in parentheses. The effects of selegiline, rasagiline and clorgyline were tested using anti-human DAT antibodies; effects of amphetamine and nomifensine were tested using anti-rat DAT antibodies. For doses of drugs see text. NT, not tested, \* $P < 0.01$ .

nomifensine or amphetamine, nor chronic treatment with clorgyline (Table 1).

### [<sup>3</sup>H]-dopamine uptake in striatal synaptosomes

No significant changes were seen in  $K_M$  or  $V_{max}$  for [<sup>3</sup>H]-dopamine uptake in synaptosomes prepared from rats treated acutely or chronically with selegiline (Table 2). Selegiline was found to be a weak uptake blocker ( $IC_{50} = 106 \pm 8.2$  µM) in comparison with its metabolites (–)-desmethylselegiline and (–)-methamphetamine ( $IC_{50}$  values  $9.37 \pm 0.08$  and  $4.51 \pm 0.05$  µM respectively). Rasagiline and its metabolite R-(+)-aminoindane were found to be without any significant effect on dopamine uptake ( $IC_{50}$  values  $386 \pm 16$ ;  $2950 \pm 155$  µM, respectively). 2-Phenylethylamine (PEA) was a potent uptake blocker ( $IC_{50} = 1 \pm 0.025$  µM), and anticipated potencies were found for nomifensine ( $0.4 \pm 0.08$  µM) and GBR-12909 ( $1.5 \pm 0.002$  nM) which served as positive controls.

## Discussion

We have found an inhibitory effect of selegiline on amphetamine-induced dopamine release in rat striatum *in vivo*. A possible explanation for this effect could be a reduction of dopamine high affinity transport. The dopamine-releasing property of amphetamine is complex, involving actions at both vesicular and membrane dopamine transporters. Amphetamine can enter dopaminergic neurons by both membrane transporter (Seiden *et al.*, 1993) and by lipophilic diffusion (Mack & Bonisch, 1979). Efflux of dopamine from the cytoplasmic compartment following amphetamine uptake is enabled by reverse transport *via* the membrane transporter. Amphetamine-induced dopamine release is dependent upon the membrane dopamine transporter since it is inhibited by uptake blockade with nomifensine (Butcher *et al.*, 1988). Release experiments *in vitro* also suggest the involvement of the membrane dopamine carrier, since nomifensine inhibits the effects of amphetamine on dopamine efflux from striatal slices or synaptosomes (Raiteri *et al.*, 1979). A recent study showed that genetic deletion of DAT in the mouse abolished the ability of systemic amphetamine ( $10$  mg kg<sup>–1</sup>) to induce efflux of dopamine into the extracellular space, as measured by both microdialysis and *in-vivo* voltammetry (Jones *et al.*, 1997).

Although selegiline was found to be a very weak uptake blocker, as reported by others (Fang & Yu, 1994) its metabolites (–)-methamphetamine and (–)-desmethylselegiline were more potent uptake blockers. It has been suggested that the short-lived inhibitory effect of selegiline on dopamine uptake after acute treatment, is the result of the action of (–)-methamphetamine, the major metabolite of selegiline (Knoll,

**Table 2**  $K_M$  and  $V_{max}$  for [<sup>3</sup>H]-dopamine uptake in synaptosomes of rats treated acutely and chronically with selegiline

	$V_{max}$ (pmol mg 5 min <sup>–1</sup> )	$K_M$ (nM)
Control	$6.17 \pm 0.32$	$57.45 \pm 9.8$
1 day selegiline	$6.85 \pm 1.5$	$69.53 \pm 20$
21 days selegiline	$5.85 \pm 1.17$	$54.35 \pm 18.4$

Striatal tissue was pooled from six rats for each synaptosomal preparation, and three experimental runs were carried out for each determination.  $V_{max}$  and  $K_M$  values were determined by Lineweaver-Burke analysis.

1987; Fang & Yu, 1994). Although we could not exclude the possibility that selegiline decreases dopamine uptake through its active metabolites, several pieces of evidence argue against this possibility: (a) blood concentrations of (–)-desmethylselegiline and (–)-methamphetamine in the region of the  $IC_{50}$  for uptake inhibition ( $9.37 \pm 0.08$  and  $4.51 \pm 0.05$  µM respectively) would not be achieved after a single administration of  $0.25$  mg kg<sup>–1</sup> s.c. selegiline; (b) Our determinations were done 24 h after the last dose of drug, and because the half-lives of amphetamines are in the region of 7 h, only very low levels of amphetamine metabolites would be expected in the blood and tissue of the rat in our studies.

Another possible explanation for the effect of selegiline to inhibit DAT activity *in vivo* is the accumulation of an endogenous factor such as PEA. The degree of MAO-B inhibition produced by a single dose of selegiline is minimal, whereas following multiple dosing, 95% of the striatal MAO-B activity is inhibited, as shown previously (Lamensdorf *et al.*, 1996). In addition, endogenous levels of PEA accumulate during chronic dosing (Paterson *et al.*, 1990). It is, therefore, apparent that marked differences exist in the brain neurochemistry between the acute and chronic situations. The mechanism whereby the acute selegiline treatment decreased amphetamine-induced dopamine release is at present not clear, and is likely to be different from that seen after chronic selegiline administration. In the animals treated chronically, endogenous PEA may mediate this effect. If PEA is responsible, it would explain why no inhibitory effect was seen in the synaptosomal preparation, since endogenous PEA would be removed during the preparation of the synaptosomes.

Amphetamine caused a reduction in DOPAC and HVA efflux into striatal microdialysate together with the increase in dopamine efflux, in agreement with findings of other workers (Zetterstrom *et al.*, 1988). This reduction in DOPAC and HVA efflux was also attenuated by the 1 day but not the 21 day

selegiline treatment. Butcher *et al.* (1988) and Zetterstrom *et al.* (1986) demonstrated that the decrease in efflux of dopamine metabolites is due to a depletion in the cytoplasmatic newly synthesized pool of dopamine. This pool is the main source for DOPAC formation and is the source of dopamine that is released by amphetamine (Zetterstrom *et al.*, 1988). However, amphetamine could also reduce the efflux of the deaminated metabolites by virtue of its ability to reversibly inhibit MAO. Since reduced efflux of the deaminated metabolites was also seen following amphetamine in mice lacking the DAT molecule (Jones *et al.*, 1997) it was inferred that the reduction in metabolites is dependent on MAO inhibition by that fraction of amphetamine which enters the neuron by the lipophilic diffusion pathway.

In the rats treated chronically with selegiline, changes in dopamine synthesis have to be considered, in addition to changes in dopamine neuronal uptake. Vrana *et al.* (1992) have shown that tyrosine hydroxylase activity was significantly reduced after chronic treatment with selegiline (0.1 mg kg<sup>-1</sup> daily for 3 and 7 days). In a previous experiment we found 30% reduction in striatal tyrosine hydroxylase activity following 21 days of selegiline treatment (0.25 mg kg<sup>-1</sup> s.c. daily; Lamensdorf *et al.*, 1997). Decrease in dopamine synthesis will deplete the newly synthesized dopamine pool, and could also explain the release of a smaller amount of dopamine by amphetamine following chronic selegiline treatment.

In our earlier study (Lamensdorf *et al.*, 1996) carried out under identical conditions to the present, the release of dopamine by a depolarizing concentration of KCl was enhanced in rats treated chronically with selegiline or rasagiline, which is compatible with inhibition of dopamine uptake (Arbutnott *et al.*, 1990). The decrease in amphetamine-induced dopamine release reported here, together with the increase in basal and KCl-induced release seen in our previous study provides strong evidence for an inhibition of dopamine reuptake by chronic selegiline treatment. The assumption that the attenuation in amphetamine induced dopamine release is due to selegiline's effect on dopamine uptake is supported by previous work by Zsilla *et al.* (1986), who found that [<sup>3</sup>H]-dopamine uptake into rat striatal slices was inhibited (by 37%) by chronic treatment with selegiline, and was transiently inhibited by acute treatment. In the synaptosomal preparation we were not able to see an effect of selegiline given to the animal previously on [<sup>3</sup>H]-dopamine uptake, possibly because the diffusion of retained small molecules such as PEA is more effective in the synaptosomal preparation than in slices.

Reduction of amphetamine-induced dopamine release was seen after both 1 day and chronic selegiline treatment, whereas increased dopamine basal extracellular level occurred only after the chronic treatment (Lamensdorf *et al.*, 1996). It is thus conceivable that the increased basal dopamine extracellular fluid level depends on both an inhibition of dopamine reuptake and a dopamine displacing effect, as is produced by PEA (Philips & Robson, 1983).

The increases in DAT levels that we observed appear to be in contradiction to the proposed reduction in functional

activity of the transporter, but activity of the transporter *in vivo* is dependent on both number of active transporter molecules as well as the presence of endogenous inhibitors, e.g. PEA. Although we found an increase in number of transporter molecules by selegiline, the newly synthesized molecules may be internalized or otherwise inactive, or their potential to enhance dopamine uptake may be inhibited by endogenous PEA. Other workers who have looked at the effect of selegiline on DAT activity have studied ligand binding. Wiener *et al.* (1989) found increased binding of [<sup>3</sup>H]-mazindol in mouse brain following long term treatment with a high dose of selegiline (10 mg kg<sup>-1</sup> i.p. daily for 4 weeks), although Yeghiayan *et al.* (1997) found no effect on [<sup>3</sup>H]-GBR-12935 binding under nearly the same conditions (10 mg kg<sup>-1</sup> for 21 days).

DAT expression was not elevated by acute treatment with a potent dopamine releaser (amphetamine) or by a potent dopamine uptake blocker (nomifensine). In addition, inhibition of MAO-B or MAO-A by rasagiline or clorgyline respectively did not affect DAT expression, and DAT was increased 1 day after a single dose of selegiline, which did not increase striatal extracellular dopamine level. Marked reductions in tyrosine hydroxylase activity by ( $\alpha$ -methyl-p-tyrosine), or increases in dopamine content by administration of 3,4-dihydroxyphenylalanine (DOPA) did not affect the binding of ligands to DAT (Moody *et al.*, 1996). The 1 day selegiline treatment which elevated DAT content in our study would be expected to inhibit only about 60–70% of the MAO-B activity (Waldmeier *et al.*, 1981), and does not significantly affect dopamine metabolism (Lamensdorf *et al.*, 1996). Therefore, the effect of selegiline on DAT expression is apparently separate from its action on catecholamine metabolism.

One possibility is that (–)-amphetamine and (–)-methamphetamine produced *in vivo* from selegiline alter the expression of DAT, since in addition to the classical effects of amphetamine on the neuronal cell, it has been shown that amphetamine can induce up-regulation of zif/268 mRNA expression in rat forebrain (Wang & McGinty, 1995). However amphetamine given in the same dose as selegiline did not affect DAT level in our study. Selegiline has been shown to alter the expression of mRNA for a variety of proteins such as tyrosine hydroxylase, L-aromatic amino acid decarboxylase and glial fibrillary acidic protein (Li *et al.*, 1992; 1993; Vrana *et al.*, 1992) in lower dosage than is needed to influence dopamine metabolism.

In conclusion, we found functional evidence for a reduction in striatal dopamine uptake *in vivo* by selegiline, together with increased synthesis of dopamine transporter molecules. The increase in expression of transporter molecules may be a property of selegiline separate from its effect on MAO. From the present results we cannot tell whether the increase in the transporter expression is due to increase in transporter synthesis or reduction in its metabolism.

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